CLAIMS

1. A method for detecting a mutation in a target nucleic acid sequence, said method comprising:

(a) providing a plurality of nucleic acid sequences comprising said target nucleic acid sequence, said target nucleic acid sequence comprising said mutation, wherein said mutation does not produce a restriction endonuclease site;

(b) providing a first primer hybridizable to said first portion of said target nucleic acid sequence comprising said mutation, said first primer comprising a second portion of said target nucleic acid sequence, and a second primer hybridizable to said target nucleic acid sequence, wherein said first and second primer comprise a polymerase chain reaction (PCR) primer pair; wherein one of the primers of this pair is designed to change the DNA sequence of the resultant PCR amplicon to produce a new restriction endonuclease site at the site of the mutation;

(c) performing a PCR amplification of said plurality of nucleic acid sequences using said PCR primer pair, wherein said PCR amplification produces a target nucleic acid sequence amplicon comprising a site recognizable by a restriction endonuclease, said site comprising the first portion and second portion of said restriction endonuclease site, said first portion and second portion of said restriction endonuclease site operably linked by said PCR amplification;

(d) incubating PCR amplicons produced in step (c) with said restriction endonuclease capable of recognizing said restriction endonuclease site under conditions permissible for said restriction endonuclease activity; and

(e) detecting nucleic acid fragments produced in step (d), wherein detection of a pattern of nucleic acid fragments is indicative of a

presence of said target nucleic acid sequence amplicon comprising a site recognizable by said restriction endonuclease,

said pattern of nucleic acid fragments being indicative of a presence of a mutation in said target nucleic acid sequence in said plurality of nucleic acid sequences.

2. The method of claim 1, wherein said target nucleic acid sequence is an allele associated with a disorder.

3. The method of claim 1, wherein said target nucleic acid sequence is an oncogene or a proto-oncogene.

4. The method of claim1, wherein said plurality of nucleic acid sequences comprising said target nucleic acid sequence is isolated from a biological sample.

5. The method of claim 4, wherein said biological sample is a patient sample.

6. The method of claim 1, wherein said presence of said mutation in said target nucleic acid sequence is indicative of a disorder or a predisposition to a disorder.

7. The method of claim 6, wherein said disorder is selected from the group consisting of a genetic disease, a metabolic disease, and hyperproliferative disease.

8. The method of claim 7, wherein said hyperproliferative disease is a cancer.

9. The method of claim 6, wherein said predisposition to a disorder is selected from the group consisting of a predisposition to a genetic disease, a metabolic disease, and hyperproliferative disease.

10. The method of claim 9, wherein said hyperproliferative disease is a cancer.

11. The method of claim 3, wherein a mutation in said oncogene is an activating point mutation.

12. The method of claim 3, wherein said oncogene is a B-RAF allele comprising a mutation or an N-RAS allele comprising a mutation.

13. The method of claim 12, wherein said mutation in a B-RAF allele encodes a mutated B-RAF polypeptide comprising a V599E mutation.

14. The method of claim 13, wherein said PCR primer pair comprises a first primer SEQ ID NO: 7 and a second primer SEQ ID NO: 8.

15. The method of claim 14, wherein said site recognizable by said restriction endonuclease is an Alw26 I site.

16. The method of claim 15, wherein said pattern of nucleic acid fragments indicative of a presence of said target nucleic acid sequence amplicon comprising a site recognizable by Alw26 I comprises fragments of 123 and 37 base pairs.

17. The method of claim 16, wherein detection of said pattern of nucleic acid fragments is a positive indicator of melanoma.

18. The method of claim 12, wherein said mutation in an N-RAS allele encodes a mutated N-RAS polypeptide comprising a Q61R mutation.

19. The method of claim 18, wherein said PCR primer pair comprises a first primer SEQ ID NO: 9 and a second primer SEQ ID NO: 10.

20. The method of claim 19, wherein said site recognizable by said restriction endonuclease is a Bcg I site.

21. The method of claim 20, wherein said pattern of nucleic acid fragments indicative of a presence of said target nucleic acid sequence amplicon comprising a site recognizable by Bcg I comprises fragments of 168, 34, and 22 base pairs.

22. The method of claim 21, wherein detection of said pattern of nucleic acid fragments is a positive indicator of melanoma.

23. The method of claim 12, wherein said mutation in an N-RAS allele encodes a mutated N-RAS polypeptide comprising a Q61K mutation.

24. The method of claim 23, wherein said PCR primer pair comprises a first primer SEQ ID NO: 11 and a second primer SEQ ID NO: 12.

25. The method of claim 24, wherein said site recognizable by said restriction endonuclease is an Sfu I site.

26. The method of claim 25, wherein said pattern of nucleic acid fragments indicative of a presence of said target nucleic acid sequence amplicon comprising a site recognizable by Sfu I comprises fragments of 210 and 40 base pairs.

27. The method of claim 26, wherein detection of said pattern of nucleic acid fragments is a positive indicator of melanoma.

28. The method of claim 1 wherein said mutation is in a BRCA 1 or BRCA 2 gene.

29. The method of claim 1 wherein said mutation is in a KRAS gene.

30. The method of claim 1 wherein said mutation is in an EGFR gene.

31. A method for detecting a mutation in a target nucleic acid sequence, said method comprising incorporating a restriction enzyme site into a target nucleic acid sequence amplicon, wherein said incorporating is effectuated by polymerase chain reaction (PCR) of a target nucleic acid sequence comprising a mutation, wherein said restriction enzyme site incorporated by PCR comprises said mutation in said target nucleic acid sequence amplicon, and said target nucleic acid sequence amplicon comprising the restriction enzyme site is digestable by said restriction enzyme, and digesting with said restriction enzyme produces a pattern of nucleic acid fragments indicative of a presence of a target nucleic acid sequence comprising a mutation.

32. A method for diagnosing a disorder associated with a mutation in a target nucleic acid sequence, said method comprising:

(a) providing a plurality of nucleic acid sequences comprising said target nucleic acid sequence, said target nucleic acid sequence comprising said mutation, wherein said mutation does not produce a restriction endonuclease site;

(b) providing a first primer hybridizable to said first portion of said target ucleic acid sequence comprising said mutation, said first primer comprising a second portion of said target nucleic acid sequence, and a second primer hybridizable to said target nucleic acid sequence, wherein said first and second primer comprise a polymerase chain reaction (PCR) primer pair; wherein one of the primers of this pair is designed to change the DNA sequence of the resultant PCR amplicon to produce a new restriction endonuclease site at the site of the mutation;

(c) performing a PCR amplification of said plurality of nucleic acid sequences using said PCR primer pair, wherein said PCR amplification produces a target nucleic acid sequence amplicon comprising a site recognizable by a restriction endonuclease, said site comprising the first portion and second portion of said restriction endonuclease site, said first portion and second portion of said restriction endonuclease site operably linked by said PCR amplification;

(d) incubating PCR amplicons produced in step (c) with said restriction endonuclease capable of recognizing said restriction endonuclease site under conditions permissible for said restriction endonuclease activity; and

(e) detecting nucleic acid fragments produced in step (d), wherein detection of a pattern of nucleic acid fragments is indicative of a

presence of said target nucleic acid sequence amplicon comprising a site recognizable by said restriction endonuclease,

said pattern of nucleic acid fragments being indicative of a presence of a mutation in said target nucleic acid sequence in said plurality of nucleic acid sequences, wherein said presence of said mutation is a positive diagnostic indicator of a disorder associated with said mutation in said target nucleic acid sequence.

33. The method of claim 32, wherein said target nucleic acid sequence is an allele associated with a disorder.

34. The method of claim 32, wherein said target nucleic acid sequence is an oncogene or a proto-oncogene.

35. The method of claim 32, wherein said plurality of nucleic acid sequences comprising said target nucleic acid sequence is isolated from a biological sample.

36. The method of claim 35, wherein said biological sample is a patient sample.

37. The method of claim 32, wherein said presence of said mutation in said target nucleic acid sequence is a positive indicator of a disorder or a predisposition to a disorder.

38. The method of claim 37, wherein said disorder is selected from the group consisting of a genetic disease, a metabolic disease, and hyperproliferative disease.

39. The method of claim 38, wherein said hyperproliferative disease is a cancer.

40. The method of claim 37, wherein said predisposition to a disorder is selected from the group consisting of a predisposition to a genetic disease, a metabolic disease, and hyperproliferative disease.

41. The method of claim 40, wherein said hyperproliferative disease is a cancer.

42. The method of claim 34, wherein a mutation in said oncogene is an activating point mutation.

43. The method of claim 34, wherein said oncogene is a B-RAF allele comprising a mutation or an N-RAS allele comprising a mutation.

44. The method of claim 43, wherein said mutation in a B-RAF allele encodes a mutated B-RAF polypeptide comprising a V599E mutation.

45. The method of claim 44, wherein said PCR primer pair comprises a first primer SEQ ID NO: 7 and a second primer SEQ ID NO: 8.

46. The method of claim 45, wherein said site recognizable by said restriction endonuclease is an Alw26 I site.

47. The method of claim 46, wherein said pattern of nucleic acid fragments indicative of a presence of said target nucleic acid sequence amplicon comprising a site recognizable by Alw26 I comprises fragments of 123 and 37 base pairs.

48. The method of claim 47, wherein detection of said pattern of nucleic acid fragments is a positive indicator of melanoma.

49. The method of claim 43, wherein said mutation in an N-RAS allele encodes a mutated N-RAS polypeptide comprising a Q61R mutation.

50. The method of claim 49, wherein said PCR primer pair comprises a first primer SEQ ID NO: 9 and a second primer SEQ ID NO: 10.

51. The method of claim 50, wherein said site recognizable by said restriction endonuclease is a Bcg I site.

52. The method of claim 51, wherein said pattern of nucleic acid fragments indicative of a presence of said target nucleic acid sequence amplicon comprising a site recognizable by Bcg I comprises fragments of 168, 34, and 22 base pairs.

53. The method of claim 52, wherein detection of said pattern of nucleic acid fragments is a positive indicator of melanoma.

54. The method of claim 43, wherein said mutation in an N-RAS allele encodes a mutated N-RAS polypeptide comprising a Q61K mutation.

55. The method of claim 54, wherein said PCR primer pair comprises a first primer SEQ ID NO: 11 and a second primer SEQ ID NO: 12.

56. The method of claim 55, wherein said site recognizable by said restriction endonuclease is an Sfu I site.

57. The method of claim 56, wherein said pattern of nucleic acid fragments indicative of a presence of said target nucleic acid sequence amplicon comprising a site recognizable by Sfu I comprises fragments of 210 and 40 base pairs.

58. The method of claim 57, wherein detection of said pattern of nucleic acid fragments is a positive indicator of melanoma.

59. The method of claim 17, 22, 27, 48, 53, or 58, wherein said detection of said pattern of nucleic acid fragments is an indicator of melanoma disease progression.

60. The method of claim 28 wherein said detection of said pattern of nucleic acid fragments is an indicator of breast cancer disease progression.

61. The method of claim 29 wherein said detection of said pattern of nucleic acid fragments is an indicator of pancreatic disease progression.

62. The method of claim 30 wherein said detection of said pattern of nucleic acid fragments is an indicator of lung cancer disease progression.

63. A method for the detection of nucleic acid fragments produced from a digestion of PCR amplicons which comprises a quantitative real-time polymerase chain reaction system.

64. A method for the detection of nucleic acid fragments produced from a digestion of PCR amplicons using a fluorophore/biotin system.